Vasoactive Intestinal Peptide, a potent stimulator of mitosis in embryos, regulates cyclin mRNA content. Stephen J. Servoss, Gordon W. Glazner, Pierre Gressens, Joyce Y. Wu, Douglas E. Brenneman, and Joanna M. Hill

Vasoactive intestinal peptide (VIP) has been shown to be a potent stimulator of growth in cultured embryonic day 9.5 (E 9.5) mouse embryos (Gressens *et al.*, Nature, 1990). VIP (10^{-7}M) treatment for 4 hours caused significant increases in DNA and protein content, somite growth, and BrDU incorporation, as well as a significant decrease in the duration of the cell cycle relative to untreated embryos. The observation that both DNA content and rate of synthesis were elevated indicates that VIP may act by stimulating mitosis in this system. The timing, duration, and stages of the cell cycle have been shown to be tightly regulated by a class of proteins termed cyclins. Given the profound effect of VIP on mitosis and cell cycle duration, we investigated the possibility that cyclin gene expression is modulated by VIP. Whole embryos (E9.5) were cultured in rat serum at 37°C, and some were treated with 10^{-7}M VIP. After 2 hours, embryos were harvested, and total RNA was extracted, quantitated, and reverse transcribed. Primer sequences were designed to be highly specific for mouse cyclins A, B and E, which are associated with G2, S, and G1/S transition, respectively. Templates were synthesized *in vitro* with identical priming sites, but of a different length, for use as cDNA mimics. Cyclin message levels were quantitated on the basis of relative [^{32}P]dCTP incorporation in a competitive PCR reaction. Total RNA levels for each embryo were quantitated, and found to be significantly higher in the VIP-treated group. Cyclin A, B, and E per embryo were increased to 236%, 243% and 263% of controls, respectively, and 167%, 184%, and 200% of controls when measured per total RNA. This significant increase in cyclin A,B, and E transcript levels is consistent with a molecular role for VIP in mitotic stimulation.

Source: https://www.industrydocuments.ucsf.edu/docs/kqcb0000